Effect of Vasectomy on the Morphology and Histochemistry of the Accessory Sex Glands of Male Rabbit

By

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To my father Juma, mother Awatif, husband Talib, sons Hazim and Amien, daughter Maria, Brothers and Sisters, whose love, care and encouragement have helped me to accomplish this work.

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TABLE OF CONTENTS

LIST OF TABLES

الخلاصة

1.اجريت الدراسات المورفولوجية، القياسية، الكيميائية والالكترونية علي غـدة الحويـصلة المنوية، غدة البروستاتا و البصيلية الاحليلية لزكور الارانب محليـة الـسلالة، وذلـك بعـد خضوعها لعملية فصل الوعاء الناقل من جانب واحد او من جانبين.

2.جمعت العينات بعد زبح الحيوانات فى اليوم ال15، 30، 60، و112 بعد العملية.

3.الغرض من التجربة دراسة الاثر الشكلى، الكيميائى والالكترونى النسيجى الناتج من عملية فصل الوعاء الناقل من جانب واحد او من جانبين على غدة الحوصلة المنوية، غدة البروستاتا و غدة البصلية الاحليلية.

4.اثر العملية يظهر فى اليوم ال60 و يصل قمته فى اليوم ال112مقارنة بالحيوانات السليمة.

5.التغيرات النسيجية اظهرت زيادة فى النسيج الضام والمحتوى الافرازى وذلك فـى اليـوم ال60، ونقصان فى حجم الحويصلات وطول الخلايا والمحتوى الافرازى فى اليوم ال112.

6.التقصى الكيميائى اظهرت زيادة فى انزيم PAS الحمضى فى اليوم 60 واليوم 112 وذلك فى غدة البروستاتا فقط، بينما لا توجد تغيير فى غدة الحويصلة المنوية والبصيلية الاحليليـة. وجد زيادة تدريجيا فى انزيم الفسفاتيز الحمضى بينما لا تغيير فى انزيم الفسفاتيز القاعدى.

7.الدراسة الالكترونية النسيجية اظهرت تباين فى شكل النواة وضمور المايتوكندريا والـشبكة الاندوبلازمية فى الحويصلة المنوية والبروستاتا، وزيادة المايتوكندريا فـى غـدة البـصيلية الاحليلية، بالاضافة الى الاجسام الالكترونية الكثيفة والفجوات الفارغة الكبيرة.

ABSTRACT

Thirty two adult rabbit's local strains were applied unilateral and bilateral vasectomy. Morphological, histometrical, histochemical and ultrastructural studies have been carried out on the seminal vesicles, prostate and bulbourethral glands of control and vasectomized animals.

The specimens have been taken after slaughtered animals on days 15, 30, 60 and 112 after unilateral and bilateral vasectomy.

The aim of the experiment was to study the effect of unilateral and bilateral vasectomy on the morphology, histometry, histochemistry and ultrastructure of the seminal vesicles, prostate and bulbourethral glands.

The effect of unilateral and bilateral vasectomy, in these glands was gave significant changes on day 60 and reached a peak on day 112, compared to the control. The histological changes demonstrated the increase in connective tissue fibers and secretory products on day 60 after vasectomy, decrease in the size of lobule and drop in cell height and secretory products in day 112 after vasectomy. These changes were approved by histometric measurement.

The histochemistry observation showing an increase in PAS- reaction in prostate gland, other what no changes in seminal vesicles and bulbourethral glands, acid phosphatase activity showing gradual intense and there were no changes in alkaline phosphatase in these glands.

Ultrastrucural studies demonstrated variation in nuclei shape, atrophy in the mitochondria and endoplasmic reticulum in seminal vesicles and prostate gland and proliferation of mitochondria in the bulbourthral glands, many electron dense bodies and large empty vacuoles.

CHAPTER ONE

1.1. Introduction

Rabbits belong to the small order lagomorphs which are distinguished from rodents (Adams 1987). This order includes the genera *lepus ochotoba* and *silvilagus*. They are classified broadly into four breeds beside some local strains (Lebas, Coudery, Rouvier and Rochambeau 1986). They are used widely in laboratories and are well suited for certain types of studies, particularly hematological investigations.

Extensive studies have been carried out on the accessory male glands of man, domestic and wild mammals and laboratory animals. (Aitken, 1959; Clegg, 1959; El –jack, 1970; Ali, 1975; Davies, Hall, Hibbitt and Moore, 1975; Abbas, 1976; Moor, Hall, Hibbit, 1976;El-sayed, 1978; Barbour, 1981; Nicaise, Lauwers and Simoens, 1991; Ahuna, Ilan, Lean, Benjamin and Joseph, 2001; Pinheiro, Almeida, Segatelli, Martinez, Padovani and Martinez, 2003).

Among the different procedures for the control of fertility in humans, vasectomy has become more popular, especially due to the simplicity of the surgical method (Castro 1988).It has been used widely for more than a century as a contraceptive method especially in the late 1960s and early 1970s, occasionally for compulsory male sterilization for eugenic reasons or as a treatment for prevention of epididymitis in prostatic surgery or, for rejuvenation (Darke, Mills and Cranston 1999).

It has been estimated that up to 100 million men world wide might have chosen vasectomy as a means of fertility control (Weiske 2001).

The effect of vasectomy on the reproductive organs has been investigated in several species including man (Neaves, 1975; Philp, Guillebaud and Budd, 1984; Jakobsen, Rui, Thomassen, Hald and Purvis, 1989; Kenneth and Moore, 1996 and Weiske, 2001), monkey (Lohiya, Tiwary, Ansari and Watts 1987 and Peng, Zhang, Dail, Deng, Wan and Yang 2002) and rat (Flickinger, 1972, Alexander; 1973 and Barrat and cohen, 1987).

1.2. Morphology:

Seminal vesicles

Seminal vesicles of man are elongated diverticula off the vas deferens at the termination of the ampullary portion, situated posterior to the prostate gland (Leeson and Leeson 1970). In stallion the seminal vesicles are paired glands, with wide central lumina which open into short branched tubuloalveolar glands (Dellman and Brown, 1981). Those of man are paired sacs or tubule-like structures, surrounded by a thick coat of smooth muscle (Carlsson, 2001).

Yao and Eaton (1954) stated that the seminal vesicles of buck are of the lobulated type and the epithelium is formed of a layer of tall columnar cells with many cuboidal basal cells. Aitken (1959) stated that the epithelium is pseudostratified and consists of a layer of round basal cells and a layer of larger, superficial cuboidal or low columnar. The epithelial lining of the secretory units of rat (Clegg, 1959), boar (Aitken,

1960), goat (Wrobel, 1970), buffalo-bull (Moussa, Badaway, Kandil and Shahin, 1983) and man (Aumuller and Riva, 1992) is simple columnar together with basal cells.

The tubules of the proximal part of the lobes are predominantly lined by simple cylindrical cells and those of the distal part by a simple squamous epithelium (Hurk, Resink and Peute, 1987). The epithelial lining of the vesicular gland of African giant rat is simple columnar and the apical surface is covered by a few short microvilli (Oke and Aire, 1997).

Aumuller and Riva (1992) stated that the interior surface of the human gland is separated by a dense system of connective tissue mesh-work that shows a different arrangement in the various portions of the gland. In the boar, the gland possesses a common connective tissue capsule and the tunica muscularis is thin .Also the intralobular septa consists of predominantly connective tissue and a few small muscle fibers and tubular lumina are wide (Dellman and Eurell, 1998).

The ducts are lined by simple cuboidal or stratified columnar epithelium (Dellman and Carithers, 1996). Dellman and Eurell (1998) described the main excretory ducts of the bull being lined by stratified columnar epithelium.

Little attention has been given to the effect of vasectomy on the seminal vesicles (Berger and Clegg, 1980; Mehranjani and Hemadi, 2007). Peng *et al*, (2002) are of the opinion that vasectomy of the *rhesus*

monkey dues not considerably affects the morphology or function of the prostate and seminal vesicles.

Prostate gland

The prostate gland plays an important role in the reproductive activity of males; for this reason, it received a special attention and has been studied in many species including man, camel, deer, pig, guinea pig, monkey, rat, squirrel, marsupial, shrew and armadillo (Flickinger, 1972; Mathur and Goyal, 1974; Ali, Moniem and Tingari, 1979; Champman and Champman, 1980; Macdonal, Hughes and Smith, 1980; Siwela and Tam, 1984; Nicaise, Lauwers and Simoens 1991; Wakui, Fursato, Nomuro, Asari and Kano,1992; Jen and Dixon, 1995; Gottreich, Hammel,Yogev and Terke, 1996 and Singh, Zhu and Handelsman, 1999).

According to Leeson and Leeson (1970) the prostate surrounds the urethra at its origin from the urinary bladder. The neck of the urinary bladder has been described to course caudally to the prostate gland and ventral to the rectum (McClure 1973).

Sisson and Grossman (1975) described the prostate gland of the bull as pale yellow in colour and consists of two parts which are continuous with each other. The prostate of the camel shows a different feature in which the cranial two thirds are almost free where as the caudal third is fused with prostatic urethra (Ali 1975).

Aitken (1960) reported that the gland of boar is covered externally by striated urethral muscle and internally by a discontinuous layer of smooth muscle. Kanwar and Sheiker (1977) described the capsule in the shrew being fibroelastic. In camel, Ali, Tingari and Moniem (1978) noted that the capsule is mainly fibromuscular and so is that of the ferret (Jacob and Poddar, 1986). In ruminant, Dellman and Eurell (1998) described the capsule surrounding the gland being formed of dense irregular connective tissue with many smooth muscle cells and striated muscle.

Fahmy and Osman (1972) studied the intralobular stroma of the gland of buffalo-bull and noted that it consists of bands of white fibrous tissue. A reticular net with well developed subepithelial elastic net has been observed in ram (Abbas 1976). There is dense connective tissue between alveoli of the prostate gland of Anatolian souslik (Cakir and Karatas, 2004).

Aitken (1960) described the prostate gland of boar as a tubulo-alveolar with simple columnar or cuboidal secretory epithelium. The epithelial lining of the secretory units of the rat prostate has been described as simple columnar (Mukerjee,1987). Dellman and Carithers (1996) stated that the prostate gland is present in all domestic mammals and that it is compound tubulo-alveolar in structure. Ali *et al* (1978) described active units of camel prostate as being lined with simple columnar epithelium together with inactive units lined with simple caboidal epithelium .Similar histological findings have been reported in the boar (Atiken, 1960), buffalo-bull (Moussa *et al,* 1983) and buck (Dellman and Eurell, 1998).

The interlobular ducts of goat are lined by low columnar epithelium (Worbel, 1972). Abbas (1976) stated that the ducts of the ram gland open into the urethral canal and the excretory ducts are lined with simple columnar epithelium. Dellman and Brown (1981) stated that the simple epithelium of mammal's prostate changes from stratified columnar to transitional epithelium toward the terminal portions of ducts.

Extensive studies have been carried out on the effect of vasectomy on the prostate gland. It was reported that the long –term of vasectomy, affected the secretory function of the gland of monkey (Lohiya *et al*, 1987) and man (Jakobsen *et al*, 1989) thus decreasing the volume of ejaculated semen. The association between vasectomy and prostate cancer as suggested by some epidemiological studies remains controversial (Weiske, 2001).

Bulbourethral glands (Cowper's gland)

The bulbourethral glands have been studied in many domestic animals (Aitken, 1960; Bharadwaj and Calhoun, 1962; Abbas, 1976; Ali *et al*, 1978 and Nogueira, Campos and Ribeiro, 1984). They are described as small paired glands located on either side of the pelvic urethra just cranial to the ischial arch but caudal to other accessory glands (Frandson, 1974). The glands of cat are located dorsolateral on either side of the distal end of the membranous urethra, which is continuous caudally as the penile urethra.

Bharadwaj and Calhoun (1962) examined the bulbourethral glands of a number of species and noted that the capsule of horse and boar is

largely fibro-elastic with some smooth muscle, whereas that of ruminants consists of dense collagenous fibers being thickest in the bull. Fahmy and Osman (1972) described a two-layered capsule in the buffalo-bull; an outer thick layer containing blood vessels and an inner layer rich in elastic fibers. Dellman and Eurell (1998) stated that the gland of the bull is ensheathed by a fibro-elastic capsule containing a variable amount of striated muscle fibers.

Aitken (1960) described the septa given off the capsule of boar bulbourethral gland indicating that they unusually contain collagenous and reticular fibers. A fibrous intralobular stroma has been described in the buffalo-bull by Fahmy and Osman (1972).

Aitken (1959) described the parenchyma of ram glands being tubulo-alveolar in structure whereas those of boar are compound tubuloalveolar in type. Fahmy and Osman (1972) described the glands of buffalo-bull as alveolar. This is essentially the same as in camel glands which are described as tubulo-alveolar (Ali *et al*, 1978).

The bulbourethral gland of man consists of short coiled tubules often dilated into alveoli and covered externally by connective tissue (Riva, Usai, Cossu, Lantini, Scarpa and Testa, 1990).

The secretory units are described as columnar with basal nuclei in sheep, goat, horse, bull, pig and cat (Bharadwaj and Calhoun, 1962 and Dellman and Eurell, 1998). Aitken (1959) described the secretory units of sheep as pyramidal with flattened basal cells but Abbas, (1976) claims

a shape of cuboidal to columnar. In the buffalo-bull the secretory units are lined with columnar or cuboidal cells (Mossa *et al*, 1983).

The duct system of the bulbourethral glands has been studied by Aitken, (1959) who claimed that the duct of ram gland is lined with transitional epithelium. Ali *et al* (1978) stated that the camel duct is lined with simple columnar but the main duct is lined by transitional epithelium. Ducts of bull are divided in two portion; initial portions lined by psedostratified cuboidal or columnar epithelium, and a terminal portion lined by stratified columnar or transitional epithelium (Dellman and Carithers, 1996).

No attention has been given to the effect of vasectomy on the morphology or function of bulbourethral glands.

1.3.**Histometry**

The histomeric investigation has been carried out on the effect of vasectomy on the seminal vesicles, prostate and bulbourethral gland of rat (Nemeth and Lee, 1996 ; Pereira, Martinez, Martinez and Mello, 2006) monkey (Peng *et al*, 2002) and man (Jakobsen *et al*, 1989).

The average heights of columnar cells of camel prostate and bulbourethral gland were 17 *n*m and 21 *n*m respectively (Ali *et al*, 1978). The height of secretory epithelium of vasectomize rat (24 hour and 7 days after bilateral vasectomy) was not statistically different from control animals (Pereira *et al*, 2006).

1.4. Histochemistry

Much work has been carried out on the histochemistry of the male accessory glands of boar (Aitken, 1960), camel (Ali *et al*, 1975), ram (Abbas, 1976), goat (Tsukise and yamada, 1984), bull (Dellmann and Eurell, 1988).

1.4.1 Periodic Acid Schiff (PAS):

The localization of periodic acid Schiff (PAS) positive reaction in the prostate, seminal vesicles, bulbourethral glands has been studied by Abbas (1976), Ali *et al* (1978), Tsukise, Sugawa and Yamada, (1979), Dellmann and Brown (1981) and Tsukise and Yamada (1984).

1.4.2. Acid and Alkaline phosphatase:

The enzymatic activity of the acid and alkaline phosphatase in seminal vesicles, prostate and bulbourethral glands, has been studied by Aitken (1960), Toner and Baillie (1966), Stallcup (1969), Abbas (1976), Ali *et al* (1978), Moussa *et al* (1983), Jacob and Poddar (1986), and Hurk, Resink and Peute (1987) in boar, mouse, bull, buck, ram, camel, buffalo-bull, ferret and African cat fish respectively. According to their studies, positive enzymatic activity has been observed in the epithelial lining of the alveoli.

Toner and Baillie (1966) reported the activity of the alkaline phosphatase enzyme principally in the stroma and basement membranes of epithelium and blood vessels of the mouse seminal vesicles. Stallcup

(1969) stated that the alkaline phosphatase activity is restricted only to the nuclei and basement membrane of the bull's bulbourethral gland.

According to Toner and Ballie, (1966) acid phosphatase activity is also readily seen in the epithelium of the mouse's seminal vesicles. Acid phosphatase enzyme is observed in the cytoplasm of the prostatic epithelium of camel (Ali *et al*, 1976; Kanwar and Sheikher, 1977). Abbas (1976) noted that the positive reaction of the acid phosphatase occurred in the basal parts of the secretory cells of the bulbourethral glands.

1.3. Ultrastrucure

The ultrastrucre of seminal vesicles and prostate gland has been extensively studied only in small laboratory animals. A number of investigations with the electron microscope have been carried out on the epithelium of the seminal vesicles of rodents (Fujita, 1959; Deane and Porter, 1960), and man (Brandes and Bourne 1954). They described the ultrastructure of man's prostate and the posterior lobe of the rabbit's prostate (Schantz, 1964).

Riva (1967) reported the presence of two types of cells; principal and basal cells in epithelium of the human seminal vesicles. The principal cells of cleanose skate(*Raja eglanteria*) are the most common, having long cilia and short microvilli and an occasional, basal cell with supranuclear accumulations of small, round mitochondria (Jones and Hamlett, 2006).

This study was therefore undertaken with the objective of examining the effect of vasectomy on the morphology and histochemistry

of the accessory glands of the male rabbit. Normal histology, histometry, histochemistry and ultrastructural observations will be compared with those of the vasectomized animals. It is hoped that this will yield some information related to function, or serve as a basis for that, and in turn, contributes to the controversy on the effect of vasectomy as a method of male contraception.

CHAPTER TWO

Material and Methods

A total number of 32 adult rabbits from local strain were used in this study. They were kept for four weeks in a farm near Shambat Campus, until they attained almost an equal weight (1Kg). They were then divided into four groups (A, B, C and D) of eight animals each; of two controls and six vasectomized.

A unilateral and bilateral vasectomy was performed. The animals were anesthetized with an intramuscular injection of (5%) diazepam (5mg\kg). After 10 minutes they were injected intramuscularlly by (5%) ketamine hydrochloride (25mg\kg), and then the animal slept (Fig 1). The hair was removed by superstainless blades employing iodine as antiseptic. They were then injected subcutaneously by (2%) lidocaine as a local anesthetic.

According to Melo *et al* (1997), (1-2 cm) abdominal vertical incision, near the linea alba, in pelvic region, was made cutting through skin, cutaneous and recti-abdominalis muscle. The spermatic cord was exposed through the incision of the tunica vaginalis (Fig 2).

The vas deferens was isolated from the testicular blood and lymphatic vessels, nerve plexus and its blood supply, using a blunt scissor. A2 loops, 0.5 cm apart, were applied on the vas deferens using nonabsorbable surgical suture (U.S.P1metric 75cm).The segment between the two loops (0.5) was excised.

For bilateral vasectomy, both spermatic cords were exposed and the vasa deferentia were treated.

The abdominal opening and the muscular layers were closed by the simple continuous suture and the skin opening closed by the loopular suture using, cat-gut absorbable suture (2metric) (Fig 3).

The control and vasectomized animals were housed and killed at (15, 30, 60, 112) days after vasectomy. Samples from seminal vesicles, prostate and bulbourethral glands were removed and placed in formalin, Boin's fluid and formal saline and processed for paraffin wax sections, fresh frozen in liquid nitrogen for histochemical study or glutaraldehyde buffer for ultrastructural observations.

2.1. Histology:

Sample of tissue of seminal vesicles, prostate and bulboureathral glands were collected shortly after slaughter of control and vasectomized animals 15, 30, 60 and 112 days after vasectomy. This procedure also applies to a- Histometry. b-Ultrastructure.

For histological observation samples (3-5mm thickness) were fixed in 10% buffered formalin solution, formal saline or Bouin’s solution. They were then dehydrated in ascending concentrations of ethyl alcohol (70%, 90% and 100%), cleared in xylene or chloroform and impregnated with paraffin wax (Drury and Wallington 1980). Sections 5-7 µm thick were cut in a rotary microtome and mounted on glass slides.

Staining:

For general histological observations, the sections were stained with haematoxylin and eosin (H & E). Special stains included;

1. Verhoeff's (1908) haematoxylin elastic tissue stain (Carleton 1967) for elastic fibers.

2. Gordon and Sweet's method for reticular fibers (Carleton 1967).

3. Van Gieson picrofuchin technique for collagenous fibers (Smith and Bruton 1978).

2.2 Histometry

Histometrical measurements were recorded for the seminal vesicles, prostate and bulbourethral glands of control and vasectomized animals, from day 15-112 after unilateral and bilateral vasectomy; to determine the cell height of the epithelial lining of the glands mentioned above, it was measured randomly from three lobules, in the field under the microscope. Olympus microscope (kc-267145- Tokyo), with ocular micrometer lens 40X was used. The objective lens 40X was used for determining the measurements, after calibrating the ocular scale of the microscope (Thienpont, Rochette and Vanparijs 1986).

Average of the epithelial height for each gland was calculated, recorded and analyzed.

2.3. Histochemistry:

Histochemical investigation were made on paraffin, fresh frozen

and fixed frozen sections.

2.3.1. Carbohydrates;

Tissue samples (3-5mm in thick) were fixed in 10% buffered formalin solution, 10%formal saline or Bouin's solution. They were then processed for paraffin sections cut at thickness of (5-7 μm) on a rotary microtome and stained by the periodic acid Schiff (PAS) technique control sections for glycogen were treated with (0.1%) amylase for 30 minuted at 37C (Carleton 1967).

Preparation of fresh frozen tissue:

Tissue samples (3-5mm thick) were freshly frozen in liquid nitrogen. Sections were cut at a thickness of (10 μm) in a Slee Cryostat, maintained at -20C, picked up on cover slips and stored in Columbia jars containing acetone. The sections were utilized for the study of the following:

2.3.2. Acid phosphatase:

The sections were fixed in acetone and stained at 37C, PH5.2 using modified Gomori's lead nitrate technique (Culling 1974).

2.2.3. Alkaline Phosphatase

The sections were stained according to the method described by Carleton (1967) and incubated at PH 9.2 for 30 mints at 37C.

2.4.Ultrastructure:

Small pieces (1x1mm in diameter) of tissue were taken from seminal vesicles, prostate and bulbourethral glands of control and vasectomized animals and fixed rapidly in 2% glutaraldehyde phosphate buffer at a PH 7.38; then changed to 5% glutaraldehyde in phosphate buffer at a PH 7.4 for 2-4 hours at 4C. The blocks were then washed every 15 minutes four times for 3o-6o minutes in phosphate buffer using Shaker machine. They were then post-fixed in 1% osmic acid for 2 hours ,and washed in phosphate buffer every 15 minutes four times for 30-60 minutes, using a shaker machine.

Dehydration was carried out in ascending grades of ethanol , 50% and 70 % alcohol over night ,90 % alcohol for 30 minutes and absolute alcohol for 30 minutes for two changes. The blocks were then immersed in propylene oxide for 30 minutes and transferred to a solution containing propylene oxide (A) mixed with solution (B) composed of (EPON 15ml ,ARALDITE 15ml and DDSA 36 ml) for 30 minutes. The ratio of A: B was 1:1. The blocks were finally left in the mixture for 2 hours. Then Dimethyl aminomethyl phenol (DMP30 1.5 %) was added to the mixture to make it hard for blocking.

The blocks were kept in an oven at 70c for 48 hours. They were trimmed on a Rechertultratrim. Semi-thin sections (0.5 µm) were cut on the ultra-tome, using glass knives, and were then stained with toluidine blue and examined with the light microscope.

The desired regions for electron microscopy were then selected and ultrathin sections, pale gold to silver (70-90 µm), were cut with glass knives. The sections were mounted on uncoated copper grids ,double stained with uranyl acetate for 5 minutes, washed in distilled water and placed in lead citrate for 30-40 seconds. They were then washed, dried and examined in a Zeiss EM 109 electron microscope (Bancroft and Stevens 1990).

CHAPTER THREE

Results

3.1. Morphological Observations

Seminal vesicles

The seminal vesicles are paired saccular gland lying caudal to the urinary bladder, dorsal to the pelvic urethra and partially covered dorsally by the peritoneum. The gland is brown in color (Fig 4).

Control Animals;

Histologically, the gland is lobulated and covered by connective tissue capsule (Fig5). The latter consists of collagenous fibers, elastic fibers, smooth muscle (Fig 6) and a few reticular fibers. Septa containing thick collagenous fibers are given off the capsule and they divide the sac into a number of lobules of different sizes. The mucosa of the lobule is folded; primary folds branch into secondary and tertiary folds that project into the lumen, which contains some densely staining masses of secretory material (Fig7). The epithelial lining is pseudostratified columnar with oval nuclei. Spherical basal nuclei have been seen in close proximity to the basement membrane (Fig 5). The intralobular ducts are lined by simple columnar epithelium.

Vasectomized Animals;

On day 15 after unilateral vasectomy, there were no significant changes in the gland. The lobule was surrounded by connective tissue

and smooth muscle fibers. The mucosa was lined by pseudostratified columnar epithelium. Secretory product was seen in the lumen. On day 30 after vasectomy, no morphological changes were noted. The lobules were surrounded by a layer of connective tissue containing smooth muscle. The mucosa was lined by pseudostratified columnar cells. There was a secretory product in the lumen.

On day 60 after unilateral vasectomy, no changes in morphological features were noted, except for an increase in connective tissue in the capsule and in the interlobular connective tissue. The luminal cytoplasm stained dark and so was the big luminal droplets; this is probably indicative of a secretory product accumulating in the tips of cells.

On day112, there was a remarkable increase in the amount of connective tissue together with a decrease in the amount of secretory product, many alveoli were completely empty.

On day 15 after bilateral vasectomy as noted in control animals, the gland is lobulated and surrounded by connective tissue capsule consisting of collagenous fibers, a few reticular fibers, elastic fibers and smooth muscle fibers. The mucosa of lobules is lined by pseudostratified columnar epithelium with spherical basal nuclei. Secretory product was seen in the lumen.

On day 30 after bilateral vasectomy, the morphological features were similar to the above. The secretory units possessed wide lumina. No changes in the amount of connective tissue of septa and capsule were noted. Secretory product was seen in the lumen.

On day 60 after bilateral vasectomy, there was an obvious increase in the connective tissue and decrease in the size of lobule (Fig8). A small amount of secretion was seen in the lumen. On day 112, there was an extensive development of connective tissue (Fig9). The lobules were smaller in size with some luminal secretion.

The prostate Gland

Control Animals;

The prostate gland surrounds the pelvic urethra immediately caudal to the urinary bladder. It consisted of an anterior part; proprostata (coagulating gland) followed by prostate and then, Paraprostate, the most caudal part (Fig4). The cranial two thirds are free and yellow in color; the caudal third is fused with the prostatic urethra, and is brown in color.

Histologically the gland is enclosed by connective tissue capsule which gives off septa that divide the gland into lobules (Fig 10), consisting of tubulo-alveolar secretory units. They rest on distinct basement membrane. The glandular parenchyma is surrounded by bundles of collagenus fibers and incomplete layer of smooth muscle fibers. The secretory units of prostate are lined by simple columnar epithelium. The terminal ducts are lined by transitional epithelium in conjunction with that of the urethra. The blood vessels and nerves are scattered throughout the stroma.

Vasectomized Animals;

On day 15 and 30 after unilateral vasectomy, there were no significant morphological changes. Units of the glandular parenchyma were surrounded by bundles of connective tissue and smooth muscle fibers. The secretory tubules were lined by simple columnar epithelium. The spherical nuclei were seen in the basal cytoplasm. Some secretory product was seen in the lumen.

On day 60, after vasectomy there was some increase in the connective tissue of capsule and septa and remarkable increase in the luminal secretion (Fig 1 1).

On day 112, there was a reduction in the size of lobules and cell height and an increase of connective tissue in capsule (Fig1 2).

On day 15 and 30 after bilateral vasectomy, there were no significant morphological or histological changes in the gland, compared with those of control.

On day 60 after bilateral vasectomy, there was a decrease in size of lobules and a drop in cell height together with an increase in the amount of connective tissue of capsule and septa (Fig 13). More secretion was seen in the lumen.

On day 112 after bilateral vasectomy, laminul secretion has disappeared in many of the lobules. Also, there was an increase in connective in the capsule and septa. Several lobules showed only minor changes with reduction in the glandular epithelial height (Fig 14).

Bulbourethral Glands

The bulbourethral glands are located caudal to the prostate gland, on the dorsolateral aspect of the pelvic urethra. They are pea-sized paired structure with a rough surface and are partially covered by the bulbocavernosus muscle. They are brown in color. The glands lie close together with a thick interglandular septum between them (Fig 4).They are about 0.7cm in length. The parenchyma is soft and its secretion is dark and viscous.

Control Animals;

Histologically, the glands are covered externally by striated muscle and internally by a thick connective tissue capsule which gives off septa dividing it into lobules (Fig15). The parenchyma consists of compound tubuloalveolar, secretory units lined by simple columnar epithelium, with basally located spherical nuclei (Fig16).

The interlobular ducts are lined by simple columnar or cuboidal epithelium. The terminal duct opens into the urethra and is lined by transitional epithelium continuous with the lining of the urethral canal (Fig17). Secretory product is seen in the lumen. The subepithelial connective tissue contains numerous large nerves and blood vessels.

Vasectomized Animals;

On day 15 and 30 after unilateral vasectomy, there were no significant morphological changes. The gland was covered externally by thick fibrous capsule. The glandular parenchyma was composed of

numerous compound tubuloalveolar secretory units surrounded by bundles of connective tissue fibers i.e. as described in the control.

On day 60 after vasectomy, no changes in the morphological features, except for some increase in the amount of connective tissue fibers of capsule and septa (Fig 18).

On day 112 after unilateral vasectomy, there was a further increase in the connective tissue, being at the expense of the glandular parenchyma which became reduced in size.

On day 15 and 30 of bilateral vasectomy no further changes have been encountered. On day 60, there was an increase in the connective tissue and lymphocytes in both lumina and stroma.

On day 112 after vasectomy, there was an abundant connective tissue fiber around the lobules (Fig19). The epithelial cells were arranged in the form of rosette-shaped alveoli. Numerous lymphcytes were seen intra epithelially of secretory units while some were seen in the capsule (Fig19).

3.2. Histometry

Seminal vesicles

The histometric analysis of seminal vesicles of the data revealed that the height of cell constituted about (12.75± 0.557)for control animals, while that of the unilaterally vasectomized animals, represented for unilateral vasectomy on day 15 after vasectomy, was about(12.69 ±

0.634), on day 30 (11.78± 0.243), on day 60 (8.85±0.04) and on day 112 (7.7±0.787).In bilateral vasectomy the cell height constituted on day 15after vasectomy about (12.33±0.296), on day 30 (10.6±0.372),on day 60 (7.32±1.37) and on day 112(5.92±0.08).There was a remarkable reduction in the cell height between the control and those of unilateral or bilateral vasectomy (Table 1).

Prostate Gland:

In the prostate gland, the cell height of gland of control animals was about (14.31±0.214), that of unilateral vasectomy on day 15 was (14.65±0), on day 30 (12.45±0.127), on day 60 (11.35±0.251) and on day112 (9.17±0.07), whereas that of the bilateral vasectomy constituted on day 15 about (14.22±0.140), on day 30 (12.46±0.262), on day 60 (11.69±0.19) and on day 112 (6.34±0.257). There were significant differences in cell heights between control and vasectomized animals; being decreased due to long term of vasectomy (Table2).

Bulbourethral glands

In bulbourethral glands, the cell height of control animals constituted about (14.79±0.28); in unilateral vasectomy on day 15 after unilateral vasectomy it amounted to about (14.54±1.067), on day 30 (11.85±0.299), on day 60 (8.41±0.633) and on day 112 (5.92 ±0.081). That of the bilateral vasectomy, on day 15 amounted to (14.45±0.098), on day 30 (11.26 ±0.151), on day 60(6.58±0.106) and on day 112 (3.72±0.111). There were no significant changes on day 15 in unilateral or bilateral vasectomy. As shown in the above figures, there is obvious

differences in the epithelial height of control and experimental animals; the latter being much lower. The difference reached a peak on day 60 and day 112 in unilateral and bilateral vasectomy (Table 3).

Table (1): Cell height (μm) of rabbit's seminal vesicles of control and vasectomized animals in period (15-112) after unilateral and bilateral vasectomy

Animal No.	Control animal	Unilateral vasectomy (day)				Bilateral vasectomy (day)			
		15	30	60	112	15	30	60	112
1	12.69	12.56	11.24	8.82	7.79	12.66	10.82	7.04	5.97
2	12.8	12.69	11.66	8.9	7.69	12.24	10.17	7.4	5.97
3	12.76	12.85	11.24	8.82	7.61	12.09	10.81	7.25	5.83
Total	38.25	38.07	34.14	26.54	23.09	36.99	31.8	21.96	17.77
Mean	12.75	12.69	11.78	8.85	7.7	12.33	10.6	7.32	5.92
S.D	± 0.557	±0.634	±0.243	±0.0.04	±0.0787	±0.296	±0.372	±1.37	±0.080

Table (2) : Cell height (μm) of rabbit's prostate gland of control and vasectomized animals in period 15-112 after unilateral and bilateral vasectomy

Animal No.	Control animal	Unilateral vasectomy (day)				Bilateral vasectomy (day)			
		15	30	60	112	15	30	60	112
1	14.08	14.65	12.30	11.09	9.24	14.36	12.75	11.68	6.47
2	14.36	14.65	12.52	11.59	9.1	14.08	12.39	11.5	6.04
3	14.5	14.65	12.52	11.38	9.17	14.21	12.24	11.88	6.5
Total	42.94	43.95	37.34	34.06	27.51	42.65	37.38	35.06	19.01
Mean	14.31	14.65	12.45	11.35	9.17	14.22	12.46	11.69	6.34
S.D	± 0.214	±0.00	±0.127	±0.251	±0.07	±0.140	±0.262	±0.190	±0.257

Table (3):Cell height (μm) of rabbit's bulbourethral glands of control and vasectomized animals in period (15-112) after unilateral and bilateral vasectomy

Animal No.	Control animal	Unilateral vasectomy (day)				Bilateral vasectomy (day)			
		15	30	60	112	15	30	60	112
1	15.07	14.65	11.88	7.68	5.97	14.51	11.09	6.6	3.84
2	14.79	14.39	12.08	8.8	5.97	14.51	11.31	6.68	3.7
3	14.51	14.08	11.59	8.75	5.83	14.34	11.38	6.47	3.62
Total	43.37	43.63	35.55	25.23	17.77	44.36	33.78	19.75	11.16
Mean	14.79	14.54	11.85	8.41	5.92	14.45	11.26	6.58	3.72
S.D	± 0.28	±1.067	±0.299	±0.633	±0.081	±0.098	±0.151	±0.106	±0.111

3.3. Histochemical Observations

3.3.1. Carbohydrates

Periodic Acid Schiff (PAS) Reaction

The Seminal Vesicles

The seminal vesicles of control animals showed PAS positive reaction in the lining epithelium of alveoli and lumina of tubules (Fig20).The connective tissue of septa and capsules gave a similarly positive PAS-reaction.

The PAS reaction in the glands of different periods after 15-112 day, of unilateral or bilateral vasectomy was almost the same.

The reaction was unaffected when the sections were treated with amylase indicating that it is not glycogen.

The Prostate Gland

A strong PAS activity of control animals was observed in some of the apical part of the columnar cells (Fig 21). PAS-positive droplets were also seen in the lumina. The capsule and blood vessels gave a weak reaction.

On day 15 after unilateral and bilateral vasectomy a strong homogenous PAS positive reaction was depicted in the columnar lining cells; the activity increased gradually on day 60 (Fig22)and intensively so on day 112.

Prior treatment with amylase resulted in the apperarance of small vacuoles.

The Bulbourethral Glands

A strong PAS positive reaction of control animals was seen throughout all lobules of bulbourethral glands (Fig 23). The terminal ducts, connective of the septa and blood vessels were moderately positive. The capsule and striated muscle gave a weak reaction.

There were no significant changes in the intensity of PAS reaction due to vasectomy of 15, 60 and 112 days (Fig24).

The reaction was unaffected when the sections were treated with amylase indicating that it is not glycogen.

3.3.2. Acid phosphatase

The seminal vesicles

In control animals, acid phosphatase activity was depicted in the epithelium. Capsule and connective tissue fibers were weakly positive to acid phosphatase enzyme (Fig25).

On day 15 after unilateral vasectomy, no change has been observed. The secretory units continued to show acid phosphatase activity. The capsule and connective tissue were weakly positive to acid phosphatase reaction. On day 60 after unilateral vasectomy, the enzymatic activity had increased and became more intense (Fig26). Capsule and connective tissue fibers showed slight enzymatic reaction. On day 112 after

unilateral vasectomy, the enzymatic activity showed even stronger reaction in the columnar cells (Fig27). Connective tissue fibers and capsule showed a poor acid phosphatase activity .The positive reaction appeared in the glands from the zero day and increased gradually with the period of vasectomy.

On day 15 after bilateral vasectomy, the cells showed a strong enzymatic reaction. A weak activity was seen in capsule and connective tissue fibers. On day 60 after bilateral vasectomy, the acid phosphatase enzyme showed very strong reaction in the cells; the capsule and connective tissue gave a poor enzymatic reaction (Fig28) .On day 112 after bilateral vasectomy, the acid phosphatase activity became intense. The enzymatic activity had greatly increased; and mainly concentrated in the cells (Fig 29). The capsule and connective tissue fibers showed a negative reaction.

Prostate Gland;

In control animals, the glandular secretory units showed strong acid phosphatase activity in the epithelial cells .The capsule and connective tissue fibers showed weak reaction (Fig30).

On day 15 of unilateral vasectomy, the acid phosphatase activity appeared similar to that of the control animals. On day 60 after unilateral vasectomy, there was a strong reaction in the epithelium which was gradually building up reaching a peak on day 112 (Fig31). A poor reaction was seen in the capsule and connective tissue.

On day 15 after bilateral vasectomy, no change has been noted, except for an increase in the amount of the enzyme on day 60 after vasectomy (Fig32). There was a gradual increase in the intensity of the reaction reaching a peak on day 112 after bilateral vasectomy (Fig 33).Acid phosphatase activity was mainly concentrated at the columnar cells .Capsule and connective tissue fibers showed negative reaction.

Bulbourethral Glands

In control animals, the glandular epithelium showed positive reaction for acid phosphatase enzyme. Weak reaction appeared in the capsule and connective tissue fibers (Fig 34).

On day 15 after unilateral vasectomy, the enzymatic reaction increased slightly in the glandular cells .Weak acid phosphatase activity has been seen in the capsule and connective tissue fibers. On day 60 after unilateral vasectomy, epithelial cells demonstrated very strong acid phosphatase activity. Capsule and connective tissue fibers showed a weak reaction. On day 112 after unilateral vasectomy, the columnar cells showed an intense reaction but it was poor in the capsule and connective tissue or poorly negative (Fig35).

On day 15 after bilateral vasectomy, the glandular cells exhibited strong acid phosphatase reaction, which increased steadily on day 60 onwards; it became intense on day 112(Fig36). Capsule and connective tissue fibers showed a weak or negative reaction.

3.2.4. Alkaline phosphatase

Seminal vesicles

In control animals, the columnar cells of alveoli showed a strong positive reaction. Weak to moderate reaction was depicted in the stroma, basement membrane and blood vessels.

On day 15, 60, and 112 after unilateral or bilateral vasectomy, no significant change has been observed, when compared to the control.

Prostate Gland

The glandular epithelium of prostate gland showed a strong positive reaction for alkaline phosphatase (Fig37). The capsule and connective tissue showed a weak reaction.

On day 15, 60 and 112 after unilateral or bilateral vasectomy, alkaline phosphatase activity was not affected qualitatively by vasectomy.

Bulbourethral Glands

In control animals, the columnar cells showed a positive reaction to alkaline phosphatase. Capsule and connective tissue showed a weak reaction (Fig 38).

In unilateral or bilateral vasectomy, on day 15, 60 and 112 after vasectomy no changes were noted.

Table (4) : Summary of histochemical observations of seminal vesicles

Substences	Control Animal	Unilateral vasectomy			Bilateral vasectomy		
		15	60	112	15	60	112
PAS	+	+	+	+	+	+	+
Acid phosphates	+	+	++	+++	+	+++	++++
Alkaline phosphates	+	+	+	+	+	+	+

Table (5) : Summary of histochemical observations of the prostate gland

Substences	Control Animal	Unilateral vasectomy			Bilateral vasectomy		
		15	60	112	15	60	112
PAS	+	+	+	++	+	++	+++
Acid phosphates	+	+	++	+++	+	+++	++++
Alkaline phosphates	+	+	+	+	+	+	+

Table (6): Summary of histochemical observations of the Bulbourethral glands

Substences	Control Animal	Unilateral vasectomy			Bilateral vasectomy		
		15	60	112	15	60	112
PAS	+	+	+	+	+	+	+
Acid phosphates	+	+	++	+++	+	+++	++++
Alkaline phosphates	+	+	+	+	+	+	+

3.4. Ultrastructrue

Seminal vesicles

In control animals, only two types of epithelial cells were identified in the seminal vesicles; principal and basal cells (Fig39). The principal cells were slender in shape with the long axes being directed towards the lumen. The apical portions looked conical or club -shaped, projecting and bulging into the lumen (Fig39).The lumen was reduced to a narrow canal with secretory canals extending between the apical parts of the principal cells. Microvillus -like cytoplasmic projections have been observed (Fig 39).

In the basal parts, cell surfaces were closely connected to one another by extensive cytoplasmic interdigitations. Clear and dense-core vesicles, indicative of adrenergic nerve endings were closely applied to the basal lamina (Fig 40).

The luminal parts of the lateral membranes of the columnar cells were joined together by junctional complexes, tight junctions (zonulae occludens), intermediate junctions (zonulae adherens) and desmosomes (maculae adherens).

The nuclei were placed at different levels in the cytoplasm; they were large and ovoid in shape. The chromatin was condensed along the inner nuclear membrane with some clumps randomly scattered in the nucleoplasm (Fig 40).

The principal cells contained a large number of mitochondria of variable sizes and were spherical or ovoid in shape. They were randomly scattered in the cytoplasm (Fig40).The cytoplasm contained also a number of free or attached ribosomes, and some smooth endoplasmic reticulum. A small Golgi apparatus was situated mainly in the supranuclear cytoplasm. Vacuoles with or without dense granules, probably secretory material, or amorphous substances were observed in the lumen (Fig 40).

The basal cells were small and setllate in shape. They were located in the angles between the bases of principal cells and rested directly on the basal lamina (Fig40). Their cytoplasm was somewhat lighter than that of the principal cells. The nucleus was spherical in shape with a moderate amount of chromatin; no nucleoli were seen. The cytoplasm contained a small number of mitochondria and other organelles and some lipid droplets.

On day 15 and 30 after unilateral vasectomy, no significant changes were noted. On day 60 after vasectomy, however, the principal cells became cuboidal in shape. The nuclei were spherical to ovoid in shape (Figs 41).More electron dense bodies appeared in the cytoplasm. Numerous vacuoles accumulated in the luminal cytoplasm; these are probably secretory products (Fig42).

On day 112 after unilateral vasectomy, the cells were slender in shape and somewhat reduced in height (Fig43). The nuclei experienced remarkable variations in shape, ranging from round, oval to kidney shape (Fig43). The nucleus looked pale with fine chromatin scattered randomly and contained one or two nucleoli. The cytoplasm contained a small

number of mitochondria and rough endoplasmic reticulum together with a moderate number of vacuoles. Basal cells were much reduced in size; their nuclei showed evidence of degeneration (Fig 43).

On days 15 and 30after bilateral vasectomy no significant changes have been observed. On day 60, there was a reduction in cell height. A remarkable number of electron dense bodies and vacuoles, many of them being empty, accumulated in the cytoplasm (Fig44).A large number of mitochondria; a small Golgi apparatus and some rough endoplasmic reticulum were observed.

On day 112 after bilateral vasectomy, the basal cells were reduced in height. There were large vacuoles similar to those described above together with electron dense granules, and heterogeneous dense bodies. Bleb-like cytoplasmic projections with vacuoles and dense material appeared pinching off into the lumen (Figs45, 46).

Prostate gland

The cells of control prostate gland were divided in two types; principal and basal. The principal cells were tall columnar. Microvilli-like cytoplasmic projections were seen on the apical surface (Fig47).There were bleb-like cytoplasmic luminal projection packed with spherical granules; these are probably secretory products. The basal parts of the lateral plasma membranes were closely held together by extensive cytoplasmic interdigitations. In the luminal parts, the lateral plasma membranes were joined together by junctional complexes, tight junctions

(zonulae occludens), intermediate junctions (zonulae adherens) and desmosomes (maculae adherens).

The nucleus was located in the basal cytoplasm (Fig47), and was generally oval or round in shape. The cells contained a small amount of mitochondria with lamellar cristae and tended to abound in the apical cytoplasm. A large Golgi complex was seen in the supranuclear region. The cytoplasm was rich in rough endoplasmic reticulum, together with some lipid droplets and numerous spherical granules in different shades of electron density (Fig 47).

Basal cells were small and stellate in shape. They were located in the angles between the bases of principal cells. The cytoplasm was relatively more dense than that of the principal cells, with a few mitochondria and some electron dense bodies and lipid droplets. The nucleus was round or oval in shape and rich in chromatin and possessed a nonucleolus. Granular or dense-cored vesicles, indicative of adrenergic nerve ending, were closely applied to the basal lamina (Fig 48).

On days 15 and 30 after unilateral vasectomy no significant change has been observed. On day 60, there was a reduction in the cell height; the cells became slender in shape with oval nuclei located in the basal cytoplasm (Fig49). The cytoplasm was packed with spherical granules of different sizes and electron opacity.

On day 112 of unilateral vasectomy, some cells looked more cuboidal in shape with round nuclei. Mitochondria and rough endoplasmic reticulum underwent atrophy. The cytoplasm was packed with spherical

granules of different sizes and electron opacity. Numerous vacuoles, with or without a small luminal content, were scattered throughout the cytoplasm (Fig 50).

On day 15 and 30 after bilateral vasectomy, no significant change has been observed. On day 60, however the cells experienced a reduction in height. The predominant feature was the massive accumulation of granules, almost masking the presence of most of other cell components (Fig 51).

On day 112, the epithelial cells were generally shorter in height than those of 60 days. Most of the rough endoplasmic reticulum and mitochondria were atrophied (Fig 52).Vacuoles and numerous small electronic dense bodies were seen throughout the cytoplasm. Massive electron-dense bodies, probably lysomes, were seen in the cytoplasm (Fig 53).

Bulbourethral glands

In control animals, two cell types were distinguished, columnar and basal. The columnar cells were more numerous, with oval nuclei occupying the basal cytoplasm. The chromatin content was concentrated heavily along the inner nuclear membrane (Fig54). The cytoplasm was rich in organelles including many mitochondria and endoplasmic reticulum (Fig54). In addition, dense membrane bound bodies were seen. The luminal border was equipped with short stunted microvilli.

The basal cells were small in size and occupied spaces between the bases of the columnar cells. The nuclei were irregular in shape.

On day 15 and 30 after unilateral vasectomy, no changes were noted. On day 60, the cells were reduced in the height. Numerous electron dense bodies and vacuoles have been seen in the cytoplasm.

On day 112, the cells became even shorter in height with the round nuclei. Although profiles of mitochondria could be observed, most of the other cell components were masked by the massive accumulation of the round dense bodies (Fig 55).

On days 15 and 30 of bilateral vasectomy, no significant changes were seen. On day 60, the cells underwent further reduction in height. A few vacuoles and numerous electron dense bodies were seen in the cytoplasm (Fig 56).

On day 112, the cells appeared much shorter with round nuclei of variable sizes. The cytoplasm contained many mitochondria and some vacuoles were randomly scattered (Fig 57, 58).

CHAPTER FOUR

Discussion

4.1. The Seminal Vesicles

The seminal vesicles of the rabbit are paired saccular structures, lying caudal to the urinary bladder, at the end of the vas deferens, dorsal to the pelvic urethra and covered dorsally by the peritoneum. This is in agreement with earlier reports for domestic animals including bull, boar and small ruminants (Sisson and Grossman 1975). According to Aumuller and Riva (1992) the gland rests on a connective tissue bed interspersed between the urinary bladder and rectum lateral to the ampulla of the vas deferens.

The capsular sheath of the gland in this study consists of connective tissue fibers with smooth muscles, blood vessels and nerve fibers. This is in agreement with Aitken, (1959) and Dellman and Eurell (1998) with regard to the gland of ram, boar and bull.

On day 60 after unilateral vasectomy, the connective tissue capsule and the interlobular septa, as seen under the light microscope, had increased in amount and reached a peak on day 112. This was also true in cases of bilateral vasectomy but the increase in the connective tissue capsule and interlobular septa was remarkable. Peng *et al* (2002) studied the *rhesus* monkey and noted that the tubular glands were sharply delineated by the muscular coat on day 180 after bilateral vasectomy.

The seminal vesicles of rabbit are tubuloalveolar glands similar to those of most domestic animals except carnivores (Moussa *et al*, 1983; Dellman and brown, 1991; Dellmann and Caithers 1996 ; Dellman and Eurell 1998). The increase in connective tissue was attained at the expense of the glandular tissue with the result of decrease in the size of lobules. This confirms the observation of Mehranjani and Hemadi (2007) who reported tubular atrophy of rat gland following vasectomy.

Among domestic animals the glandular epithelium is described as simple columnar together with basal cells as in the boar (Atiken 1960), goat (Wrobel 1970) and buffalo-bull (Mossa *et al* 1983). But in the barrows however, it is pseudostratified with low columnar superficial cells and ovoid basal cells (Lauwrs *et al* 1984). In the present study, the epithelial lining of the secretory units was pseudostratified columnar epithelium. The interlobular ducts are lined by simple columnar epithelium.

In unilateral or bilateral vasectomy, on day 15 up to day 30, no changes were encountered in the secretory cells. The changes ensued on day 60 being characterized by a lot of secretion in the lumen. On day 112 after unilateral vasectomy, there was a reduction in the height of the columnar cells. The reduction was remarkable on day 112 after bilateral vasectomy and was followed by a similar reduction in the amount of luminal secretion.

The present study demonstrated the presence of PAS-positive reaction resistant to treatment with salivary amylase. The reaction was in the form of granules in the secretory units and apparently it is not

glycogen. Similar observations were reported for the seminal vesicles of boar (Aitken 1960) and buffalo-bull (Moussa *et al* 1983). The reaction had not been affected by vasectomy.

Acid phosphatase activity demonstrated in the seminal vesicles of the normal rabbit is similar to that reported for the mouse (Toner and Ballie 1966). In the present study the enzyme activity showed a steady increase until day 112 after both unilateral and bilateral vasectomy. Toner and Ballie (1966) reported similar observation on the seminal vesicles of castrated mouse. It is tempting to assume that there are common roles played by both testis and fluids contained within its excurrent ducts. Accordingly, with the elimination of this fluid due to vasectomy, or castration, some target organs are affected. The effect due to castration is probably faster than that due to vasectomy.

The ultrastructural examination of the principal cell of the epithelium of the seminal vesicles revealed the presence of numerous irregular dense bodies, as observation which was also reported in the rat (Allison 1964; Orlandini 1964, 1966). These bodies became more numerous and larger in size on day 60 after unilateral and bilateral vasectomy onwards. This was also true of the mouse 4 weeks (Deane and Porter 1960) and 5 days (Toner and Ballie 1966) after castration. The presence of the irregular bodies in the principal cells, coupled with the demonstration of acid phosphatase in these cells, is suggestive of their nature as lysosomal bodies possibly dealing with the excessive secretion accumulating in the cells.

Alkaline phosphatase appeared in the basal cytoplasm of the lining cells of secretory end-pieces. This is in accord with reports on the gland of boar (Aitken 1960), ram (Abbas 1976) and buffalo-bull (Moussa *et al* 1983). The alkaline phosphatase enzyme was unaffected by vasectomy. Likewise, such enzymatic activity in the glands, of the castrated mouse was unaffected (Toner and Ballie 1966).

4.2. **The Prostate Gland**

The prostate gland of rabbit is located in the lamina propria of the pelvic urethra just caudal to the urinary bladder and encircles the urethra. It consists of proprostata (anterior) followed by prostata and paraprostata.

No change in shape due to vasectomy has been observed. This is in agreement with what has been reported on the glands of monkey by Kenneth and Moore (1996) and Peng *et al* (2002).

Histologically, the present study has shown that the prostate gland is branched tubular in structure with dense septa of connective tissue separating them. The septa consist of bundles of collagenous, elastic and reticular fibers and an incomplete layer of smooth muscle fibers surrounding the gland. Blood vessels and nerves were observed throughout the gland. Yao and Eaton (1954), Worbel (1972). The secretory units of the prostate gland, demonstrated in this study are lined by simple columnar epithelium.

There was no change in the epithelial height of secretory units until day 60 of bilateral vasectomy and day 112 of unilateral vasectomy. Beyond these dates, steady reduction in the epithelial height as well as the

secretory activity was encountered; a peak was reached on day 112 after bilateral vasectomy. Jakobsen *et al* (1989) dealing with man after 8 years of vasectomy reported a reduction in the secretory function. Also Melo *et al* (1997) described the epithelium of secretory units of the rat on days 60,120,180 and 360 after bilateral vasectomy as cuboidal in shape with round nucleus in the basal cytoplasm. Furthermore, it has been reported that long-term vasectomy affected the secretory function of the prostate of monkey (Lohiya *et al,* 1987) and man (Jakobsen *et al,* 1989) thus decreasing the volume of the ejaculate.

The present study demonstrated PAS-positive reaction in the alveoli of prostate gland of rabbit; similar observations have been reported in prostate gland of several species of domestic animals (Aitken, 1960; Wrobel, 1970; Ali *et al*, 1978; El-sayed, 1981; Tsukise and Yamda 1984; Kawakami *et al,* 1991).

The PAS positive reaction continued throughout all periods of vasectomy but a peak of intensity was attained on day 112 of both unilateral and bilateral vasectomy.

The enzymes acid and alkaline phosphatases were demonstrated in the control and vasectomized animals throughout all period of vasectomy. The enzymic reaction was mainly in the columnar cells of alveoli, and was weak or negative in the connective tissue and capsule. According to Aitken (1960), Stallcup (1969), Worbel (1972), Ali *et al* (1976) and Kanwar and Sheikher (1977) alkaline phosphatase activity lies in the basal

part of secretory units and acid phosphatase activity in the apical part of the secretory units.

In this study, the enzymatic reaction increased on day 60 after unilateral or bilateral vasectomy and reached a peak on day 112 after unilateral or bilateral vasectomy.

Ultrastractural examination of section of the prostate gland showed the presence of two types of cells; principal and basal. The principal cells are tall columnar with microvilli-like cytoplasmic projections on the apical surface. Mitochondria, rough endoplamic reticulum, free ribosome and dense bodies were randomly scattered in the cytoplasm.

The basal cells were located between the bases of columnar cells, and contained some lipids droplets.

Cells of the glands of the vasectomized animals on day 15 and 30 after unilateral and bilateral vasectomy were morphologically similar to those of the control. On day 60 after bilateral vasectomy and on day 112 after unilateral vasectomy, the columnar cells were somewhat cuboidal in shape with round nucleus in basal cytoplasm. This confirms the observation of Melo *et al* (1997) who described a cuboidal shape of rat cells after 120 of bilateral vasectomy. On day 112 after bilateral vasectomy performed in this study, there was a decrease in the mitochondrira, Golgi apparatus and rough endoplasmic reticulum and an increase in number of large vacuoles and electron dense bodies. This is in agreement with the report of Melo *et al* (1997) on prostate gland of rat on day 120 after bilateral vasectomy and Dahl and Kjaerhem (1973) of

prostate gland of rat on 10 days after castration.

The histochemical localization of acid and alkaline phosphatase in the cells of the prostate gland, and the demonstration of electron dense bodies in the same cell of prostate gland, as was also shown in the seminal vesicles, is again suggestive that these bodies are lysosomal in nature; dealing with excessive secretion accumulating in the cells.

4.3. The bulbourethral glands

The bulbourethral glands in this study appear as pea-sized paired structures with a rough surface and partially covered by the bulbocovernousus muscle. The glands are located caudal to the prostate gland and dorsolateral to the pelvic urethra. This is in accord with the description of the gland in many domestic animals (Frandson, 1974; Sisson and Grossman, 1975; Ali *et al,* 1978; Banks, 1993; Dellman and Eurell, 1998).

According to this study, the capsule of the bulbourethral glands consists of connective tissue fibers with bundles of smooth muscle and is surrounded externally by striated muscle. In ruminant, the capsule consists of dense collagenous fibers, being thicker in the bull (Bharadwaj and Calhoun, 1962). Dellman and Brown, (1981) reported that the exceptionally large bulbourethral gland of boar is surrounded by the glandular muscle, with a few smooth muscle fibers in the interstitial tissue. Dellman and Eurell, (1998) described the glands of bull as ensheathed by a dense white fibrous connective tissue capsule containing a variable amount of smooth muscle cells and surrounded by striated muscle similar to those of glands of rabbit.

In vasectomized animals, on day 15 and 30 of unilateral or bilateral vasectomy, there were no significant changes in the capsular layer. But on day 60 however, in both unilateral and bilateral vasectomy, there was an increase in the connective tissue fibers in the capsule and septa. On day 112 after unilateral vasectomy**,** there was a steady increase in the connective tissue which became maximal on day 112 after bilateral vasectomy. Consequently, the lobules became small in size and so were the secretory and the secretion therein; after long term of vasectomy, the secretion disappeared, i.e. the secretory units underwent atrophy.

The present study demonstrated that the glands are tublualveolar in type. The secretory units are lined by simple columnar epithelium, with basally located spherical nuclei. The interlobular ducts are lined by simple columnar and cuboidal epithelium. The terminal ducts open in the urethra and are lined by transtitisonal epithelium. In the domestic animals, Bharadwaj and Calhoun (1962) and Dellman and Eurell (1998) described the epithelium of the secretory units of the bulbourethral glands as simple columnar with basal nuclei. An epithelial lining of cubical to columnar cells was described in the ram (Abbas, 1976), camel (Ali *et al*, 1978) and buffalo-bull (Moussa *et al*, 1983).

The present study has shown the PAS-positive reaction was stronger in the secretory units, interlobular ducts and luminal contents of the bulbourethral gland. It was moderate in the connective tissue of the capsule and septa. This is in agreement with the report on the gland of camel (Ali *et al* 1978), rat (Tsukise *et al*, 1979) and bull (Dellman and Eurell, 1998). Search in the literature revealed no report on the effect of vasectomy on the PAS reaction in the bulbourethral glands of domestic or

laboratory animals. In this investigation no changes due to unilateral or bilateral vasectomy have been observed in the PAS reaction in the bulbourethral gland of rabbit.

The acid and alkaline phosphatase granules were depicted in the cytoplasm of the bulbourethral glands. Acid phosphatase was demonstrated in the apical parts of cells. The concentration of acid phoshpatase increased on day 15, 60 gradually and became more intense on day 112 after unilateral and bilateral vasectomy. Alkaline phosphatase reaction was seen in the basal parts of the cytoplasm. The intensity of the reaction remained unaltered regardless of the post-vasectomy period.

Ultrastruclural study revealed the presence of two types of cells; columnar and basal. The columnar cells were more numerous with electron dense bodies, and many membrane-bounded secretory granules. This is in agreement with similar observations which have been reported in the bulbourethral gland of rat (Nielsen 1976). Electron dense bodies, bounded or unbounded by membrane similar to those described by Nielsen (1976) were seen in the lumen. These possibly represent secretory products and\or lysomal bodies.

The basal cells were few in number being located at the bases of columnar cells. The cytoplasm was denser and the nucleus was rich in particulate chromatin granules.

In the present study, on day 15 and 30 after unilateral or bilateral vasectomy, there were no significant changes observed. On day 60 there was a reduction in the cell height, which continued with a parallel increase

in the amount of mitochondria, vacuoles and electron dense bodies on day 112 onwards.

In conclusion, it seems obvious, that there are significant changes in the cytoplasmic components of cells of bulbourethral gland (mitochondria, vacuoles and electronic dense bodies) due to long term of vasectomy.

Summary:

1- Morphological, histometrical, histochemical and ultrastructural studies have been carried out on the seminal vesicles, prostate and bulbourethral glands of 32 adult rabbit's of local strain. The specimens were obtained from normal rabbits as well as from those which underwent unilateral and bilateral vasectomy.

2- Samples were collected from slaughtered animals after different periods post vasectomy, on days 15, 30, 60 and 112 processed for histological, histometrical and ultrastructrual studies.

3-Other specimens were taken on days 15, 60 and 112 post vasectomy and processed for histochemical studies.

4- Morphologically, the accessory male glands of rabbit (seminal vesicles, proprostata, prostate paraprostata and bulbourethral glands), resembled those of other domestic animals. The following was noted:

a- Effect of vasectomy was observed on day 60 in all three glands, manifested by an increase in connective tissue and luminal secretion. A steady increase in connective tissue continued and became remarkable on day 112 with a drop in secretory activity; some alveoli were empty. The lobules became smaller in size and possibly underwent atrophy. The effect due to bilateral vasectomy exceeded that of unilateral vasectomy. There was also a drop in the epithelial height as confirmed by the histmetric measurements.

b- The histochemical investigation demonstrated the localization of PAS, in the epithelial lining of all three glands. It was unaffected by vasectomy except in the prostate gland in which it became gradually stronger and reached on intensity on day 112.

c- The reaction due to acid phosphatase was increased and maximal intensity was reached on day 112after unilateral or bilateral vasectomy. Alkaline phophatase was unaffected by vasectomy.

4- The ultrastructrue observations of the male accessory glands revealed the presence of the two types of cells, principal and basal. The effects of vasectomy on these cells are;

a- On day15 and 30, there were no changes in the cells.

b- On day 60 after vasectomy the principal cells became shorter; there are more electron dense bodies in the cytoplasm. There are secretory products in the lumen.

c- On day 112 after vasectomy, the seminal vesicles and prostate gland underwent atrophy as shown by the change of shape of mitochondria and the endoplasmic reticulum, numerous large empty vacuoles, variations in the nuclear shape and accumulation of dense bodies. In the bulbourethral gland however there was an increase in mitochondria.

Conclusion:

1. Vasectomy has an adverse morphological and possibly functional effect on the accessory male glands as manifested by the excessive deposition of connective tissue and decrease or drop in the cell height (atrophy) of the secretory units. Such effects occur on day60 post vasectomy and steadily increase to reach a peak on day 112. Bilateral vasectomy was more effective than unilateral vasectomy.

2. The appearance of the ultrastructural dense heterogenus bodies in the cytoplasm of the lining cells of secretory units, coupled with the histochemical demonstration of acid phosphatase in these cells, and its intensity with long period of vasectomy, is suggestive of these bodies being lysosomal in nature dealing with the secretory products accumulating, also in the cytoplasm.

3. The increase in the amount of mitochondria in the bulboureathral glands is puzzling. The accumulation of the interlobular connective tissue could be a mechanical impediment on the alveolar cells, and hence more energy (mitochondria) will be needed to assist secretory activity on the alveolar cells to exert more effort to secrete, and therefore more mitochondria. This may be reasonable when we consider that the secretion of bulbourethral glands is a pre-ejaculate.

ملخص الأطروحة:

1. أجريت الدراسات (المجهرية المورفولوجية، القياسية النسيجية الكيميائية النسيجية، والتركيبية الالكترونية الخلوية المجهرية) على غدة الحويصلة المنوية، البروستاتا، البصيلية الاحليلية على 32 من ذكور الأرانب البالغة محلية السلالة قسمت الى اربع مجموعات (ا،ب،ج،د) فى كل اثنين سليمة و ستة خضعت لعملية فصل الوعاء الناقل من جانب واحد أو جانبين.
2. أخذت العينات من الحيوانات بعد ذبح الحيوانات المعالجة و الحيوانات السليمة للمقارنة:

أ. في اليوم 15، 30،60 و112 لإجراء الدراسات النسيجية الشكلية، النسجية القياسية والتركيبية المجهرية الدقيقة.

ب. في اليوم 15،60و112 لإجراء الدراسة الكيميائية النسيجية.

3. شكل الغدد الجنسية الاضافية لذكر الأرانب هو الحويصلة المنوية، البروستاتا (قبل البروستاتا، البروستاتا، بعد البروستاتا) والبصيلية الاحليلية يشابه غيره في الحيوانات الأليفة الأخرى.

وهذا يتبع الملاحظات الآتية:

1. في اليوم 60 بعد عميلة فصل الوعاء الناقل اظهرت الدراسة زيادة في النسيج الضام وزيادة في كمية الافراز. زيادة كمية النسيج الضام استمرت وأصبحت واضحة المعالم مع انخفاض في النشاط الافرازي ووجود بعض الحويصلات فارغة من المادة الافرازية، صغر حجم الفصيصات ومحتمل وقوع الضمور. عملية فصل الوعاء الناقل من جانبين ذات أثر أكبر من فصله من جانب واحد.
2. إجراء القياسات النسيجية أثبتت وجود نقص فعلي قياسي في طول الخلايا نتيجة لعملية فصل الوعاء الناقل.

3.الفحص الكيميائي النسيجي أثبتت موضعية تفاعل PAS في الطلائية المحيطية للخلايا في الغدد الثلاثة وهو لا يتأثر بعملية فصل الوعاء الناقل ما عدا في غدة البروستاتا الذي يزيد فيها التفاعل تدريجياً مع طول فترة العملية ويصل قمته في اليوم 112.

4. الغدد الجنسية الإضافية لذكر الأرانب تظهر استجابتها لأنزيم الفسفاتير الحمضي والقلوي. لكن في انزيم الفسفاتير الحمضي يزداد معدل التفاعل تدريجياً بطول فترة العملية ويصل الحد الأعلى في اليوم 112. بعد عملية فصل الوعاء الناقل من جانب أو من جانبين.
5. الفحص الخلوي الالكتروني لغدة الحويصلة المنوية والبروستاتا والبصيلية الاحليلية أظهر نوعين من الخلايا: الأساسية والقاعدية. اثر عملية فصل الوعاء الناقل في هذه الخلايا تظهر في:

أ. اليوم 15-30 لا يوجد تغيير واضح في التراكيب المجهرية الداخلية.

ب. اليوم الـ 60 تصبح الخلايا الأساسية مكعباتية الشكل وتحتوى على كمية من الاجسام الالكترونية الكثيفة في السابتوبلازم ووجود افراز في التجويف.

ج. اليوم 112 هناك ضمور في في المايتوكوندريا والشبكة الاندويلازمية، فجوات فارغة كبيرة وتباين في شكل النواة وتجمع الأجسام الكثيفة الالكترونية في السايتوبلازم. غير أن في البصيلية الاحليلية توجد زيادة في كمية المايتوكندريا بالإضافة إلي التغيرات الاخرى.

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